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Research Trends: Bioactive Metabolites of Fungal Origin

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Abstract: The need to combat human, agronomic and veterinary diseases or pests has sustained research interests directed at finding new medications from under-exploited biodiversity. The adoption of bioassay techniques, which correlate with prevention, inhibition and reversal of diseases or elimination of pests in screening natural products for bioactive compounds, is critical to the discovery of new medications. This study focuses on the chemistry and biological activity of fungal secondary metabolites that connect academia with industry. It discusses the technical skills needed to find biologically active compounds from fungi.

Key words: Agronomy, biodiversity, prevention, metabolites, Nigeria

INTRODUCTION

Phytotherapy is a term derived from two words; *phyto* meaning plant and *therapy* which has to do with treatment. Hence, phytotherapy refers to the treatment of diseases using herbs or drugs of plant origin. The use of plants as medicines goes back to early man. Plants continue to be used world-wide for the treatment of disease and novel drug entities continue to be developed through research into their constituents. Despite the massive arsenal of clinical agents developed by the pharmaceutical industry, there has been an aversion by many members of the public and herbal remedies proved to be popular as alternative or complementary treatments of disease. In the developing countries large numbers of the world's population are unable to afford pharmaceutical drugs and they continue to use their own systems of indigenous medicine that are mainly plant-based. Hence, the strong need to harness scientific and clinical research in order to investigate the quality, safety and efficacy of these herbal therapies. There are three main reasons for scientific investigations in the area of natural products. They are; 1) to find biologically active compounds. Research plants or organisms are approved for bioactivity-driven isolation and identification of secondary metabolites if initial extracts (of higher plants and broth) showed useful biological activity. 2) To screen transgenic organism for a single metabolite of commercial importance. This is a natural extension of the first objective with biotechnology skills playing a major role. 3) For re-classification of a confused phylogeny. Secondary metabolites from each species of a genus are elaborately isolated, characterized and compared with one another (Kopcke *et al*., 2002).

In Nigeria, as in many other parts of the world, traditional medicines based mostly on medicinal plants have been used for the treatment of various ailments. The question is whether such anti-infective properties are produced by the plant itself or as a consequence of a mutualistic relationship with beneficial organisms in plant tissue. There is a basic supposition that any plant possessing clinical effectiveness must contain an active principle which can completely replace the plant extract. But many reports have shown that in a microbe-plant relationship, endophytes contribute substances that possess various types of bioactivity, such as antimicrobial and pesticidal (Rodriguez *et al*., 2000; Filip *et al*., 2003; Taechowisan *et al*., 2005). Hence, the interest for endophytic fungi as potential producers of novel biologically active products has increased in the last decade. Endophytes are microorganisms that live in the intercellular spaces of stems, petioles, roots and leaves of plants causing no discernible manifestation of their presence and have typically gone unnoticed.

In the last decade, the interest for endophytic fungi as potential producers of novel, biologically active products has increased. Most terrestrial plants are colonized by one or several species of endophytic fungi which can be isolated from healthy-looking host tissue and do not cause visible symptoms of infection and disease (Radu and Kqueen, 2002). Biologically active secondary metabolites are produced by many endophytes and may be involved in defining the endophyte-host plant relationship.

The nature and biological role of endophytic fungi with their host plant is variable. Endophytic fungi are known to have mutualistic relations to their hosts, often protecting plants against herbivory, insect attack or

tissue invading pathogens (Vinton *et al*., 2001). In some instances, the endophyte may survive as a latent pathogen, causing quiescent infections for a long period and induce symptoms only when physiological or ecological conditions favor virulence. For instance, while *Phoma medicaginis* can exist as a prolonged asymptomatic infection of its host plant (*Medicago* sp.), this fungus produces detectable levels of the toxin brefeldin A only during and after the switch from the endophytic to the saprobic phase upon host death (Weber *et al*., 2006). Accordingly, some endophytes could be reliable sources of materials of the agricultural and pharmaceutical potential as exemplified by taxol, subglutinol A and B and peptide leucinostatin A; all of which could be produced by both endophytes and the hosts. Recently, more interest has been generated around studying biologically active metabolites from higher fungi (Basidiomycotina), bacteria, filamentous fungi from marine habitats and symbiotic lichens. A wide array of mushrooms is known to produce antibiotics. Puffballs have been used in folkloric medicinal practices to treat sores, bruises, deep cut, hemorrhage as well as urinary tract infections (Buswell and Chang, 1993), although such claims are still to be proved scientifically.

Even if the metabolites produced by the organisms do not depict exceptionally high biological activity, they are still of inestimable value, as they occasionally display new chemical lead structures from which many new compounds may be deduced. When used as crop protection agents, they are environmentally friendly. In addition, their biodegradation by soil microbes and re-integration into the environment are readily achieved.

The biodiversity of fungi is immence. Out of the estimated 1.5 million species of fungi recorded worldwide (Hawksworth, 1991), approximately 4,000 secondary metabolites of fungal origin are known to possess biological activities (Dreyfuss and Chapela, 1994) the vast majority coming from the species of *Penicillium, Aspergillus, Acremonium and Fusarium.* The tropical regions host more than half the number of living species worldwide. Hence, a large number of biologically active metabolites is probably produced in these ecosystems. This is also supported by the fact that several tropical flora are known to possess medicinal properties and are now actively investigated by ethnobotanists.

Bioactive metabolites from extremophilic fungi: Fungi that love moderate environments are called mesophiles. Several of them are culturable. Mesophilic soil fungi have been and remain a major source of novel microbial metabolites. Over the past three decades, scientists have collected and identified fungi that flourish in extreme environments. They include fungi from hot (thermophiles, e.g., from hydrothermal vents), cold (psychophiles, e.g., from The Antarctica), acidic (acidophiles, e.g., from volcanic pools, abandoned coal mines or sour petroleum wells), salty waters (halophiles, e.g., from salty water of the Dead sea in the Middle East), alkaline pools (alkalophiles, e.g., from Kenya soda-filled Lake Magadi), high pressure regions (barophiles, e.g., from ocean floor) and from niches with above normal level of radiation. Most extremophiles are difficult to culture, yet they contain genetic materials that could code for and produce secondary metabolites that are medicinally useful. A number of them produce enzyme that have found applications in the biodegradation of chemical warfare agents (Mulbry and Rainina, 1999). Roughly one-third of drugs come from 5% of microbes that can be cultured.

Screening of fungi for bioactive metabolites: The discovery of bioactive compounds usually begins with isolation and identification of the microbes and growing them at various temperature regimes in a variety of selective and non-selective culture media. The chemicals they produce are scaled-up by fermentation. The fermentation broth is extracted, fractionated, tested and purified. Testing is usually performed using the traditional whole organism based dilution, diffusion and bioautographic assay methods. These methods have been adequately reviewed by Rios *et al*. (1988).

In recent years, receptor-based *In vitro* assays have become increasingly popular for screening fermentation broth in the industry. As an illustration, Merck scientists isolated the fungus *Psuedomassaria* from a plant collected from a forest near Kinshasa in the Democratic Republic of Congo. Its fermentation broth was extracted, fractionated and screened against a receptor-based assay, which monitors agents that interact with and activate the insulin receptors. This led to isolation of compound L-73281 with insulin-like properties (Zhang *et al*., 1999). Receptor-based assays allow the design of cellular high through-put screening procedures. They are often expensive, sophisticated, specific, automated and may require in several cases a dedicated research technician.

The hitherto common practice of phytochemists to isolate, characterize and publish phytocompounds without regard to bioactivities is no longer attractive to academic journals and funding agencies. Three techniques are required for sourcing bioactive compounds of fungal origin. They are separation methods, structural elucidation and bioassay methods. Bioassay technique may be grouped into two; the *In vivo* model that involves the use of whole or target organisms

and the *In vitro* model that is enzyme, receptor, mode of action or mechanism-based. *In vitro* model is used as an ancillary screen to supplement primary *In vivo* screens. In the domain of pharmacology, a desired objective is to extrapolate data from *In vitro* experiments to *In vivo* models.

Many discoveries in the 1980s relied on *In vivo* models that are known to be less costly, easier to perform and more adaptable for natural product screenings. Tests on whole organisms by nature are broad-based and embrace multiple targets, unlike tests on *In vitro* models, which are target-specific. The metabolites they produce can be manipulated by changing the composition of the growth media and combinatorial biosynthesis. Fungal metabolites are a complex library of chemical compounds which are structurally and pharmacologically dissimilar (Kopcke *et al*., 2002; Weber *et al*., 2006). Many potential useful compounds in fungal extracts are not detected in *In vitro* screens because the assays are too specific or because of interactions that occur among the components of extracts. It is thus a standard practice to subject extracts to chemical or biological dereplication protocols to eliminate compounds that could interfere with assay results. Solid phase extraction cartridges, direct chromatography on Sephadex G-25 or polyamide columns are used for dereplication of some compounds in fungal extracts (Taechowisan *et al*., 2005). Whatever model is adopted, screening methods must be sensitive, use a small amount of test materials, allow simple test sample preparation, give good results, have good throughput capacity and a wide field of view. The assay output must correlate with an important function in disease etiology or microbial pathogenicity.

Some antibiotic agents from fungi: Most of the common antimicrobials are obtained form microorganisms, including fungi. Consequently, several research centers and pharmaceutical industries are screening broths and extracts of fermentation in a battery of assays, searching for natural products that could conquer new or re-emerging infectious diseases. Camptothecin, isolated from endophytic fungi growing on *Mappia foetida* in India, offers high potential in cancer therapy (Spiteller and Qazi, 2006). Cyclopaldic acid from *Aspergillus duricaulis* also inhibit the growth of *Bacillus subtillis* and *Sreptomyces viridochromogenes* in agar medium. Investigations on *Colletotrichum* sp. a fungal endophyte of *Artemisia annua* (Lu *et al*., 2000), led to the islation of five ergosterol derivatives all of which were effective against *Bacillus cereus*, *Staphylococcus aureus*, *Sarcina lutea*, *Pseudomonas* sp. and some fungal phytopathogens. Fractions from crude extracts of *Aspergillus fumigatus, Guignadia* sp. *Phomopsis* sp. *Pestalotiopsis guepinii* and mushrooms-*Lycoperdon pusilum* and *L. giganteum* are known to exert effect on some Gram+ and Gram-bacteria *In vitro* (Rodriguez *et al*., 2000; Jonathan and Fasidi, 2003; Furtado *et al*., 2005). The mode or mechanism of action of these bioactive metabolites is hitherto, uncertain. But elsewhere antibiotics such as penicillins, cephalosporins and vancomycin, which are also microbial products, exert their antibiotic effects by interfering with the biosynthesis of cell wall.

CONCLUSION

A plethora of biologically active metabolites has isolated within the last Fifty years from micro-and macro-fungi. Majority of such metabolites have occupied a commanding position among drugs that are now used against agronomic pests, infective organisms, parasites of livestock and humans and diseases such as cancer and malaria. In traditional natural products screening programs, extracts that are 'hits' in a screen of interest require follow-up analysis, typically involving analytical chemists. The potential for discovering addition compounds from fungi in ecologically stressed terrestrial and aquatic environments is enormous. In addition, patent protection of novel, applicable and unobvious discoveries is necessary if the exploitation of our biota is to yield financial return, which is generally a necessary requirement to facilitate more advanced development of lead molecules. There is also an urgent need to intensify investigations towards the Nigeria mycobiota as a source of bioactive compounds, considering its biotic richness due to the high biological diversity.

Current research activities: Screening of a large collection of endophytic fungi (about 230 isolates) for biological activities.

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